A potential Role of Metformin in Hepatocellular Carcinoma: An In Vitro Study of LncRNA-AF085935/Glypican-3/MAPK Pathway

Radwa Taha¹, Omayma A. Elkholy¹, Dina Sabry¹,², Mona Said¹ and Naglaa F. Abozeid¹*

¹Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Egypt.
²Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Badr University, Cairo, Egypt.

*E-mail: naglaafathy@kasralainy.edu.eg

ARTICLE INFO

ABSTRACT

Background: Hepatocellular carcinoma is on the rise globally. The anticancer effects of metformin have been mainly studied in the context of suprapharmacological concentrations (>1 mM). In this study, we aim to investigate the effects of therapeutic doses of metformin on the LncRNA-AF085935/glypican-3/mitogen-activated protein kinase pathway in HCC.

Methods: HepG2 cells were treated with various concentrations of metformin (0, 5, 10 and 20μM) for different durations (24, 48, and 72h). Cell viability was evaluated by MTT assay, while gene expression levels of LncRNA-AF085935, glypican-3, VEGF, P53, NFκB genes were evaluated by quantitative real-time PCR. The ratio between the phosphorylated p44/42 and the total p44/42 protein level was assessed by western blot.

Results: Compared to the control groups, we found a significant decrease in cell proliferation, LncRNA-AF085935 gene expression (p value <0.001), VEGF gene expression (p value <0.001) and p-p44MAPK protein level (p value <0.0001) in all metformin-treated groups except the metformin 5μM-24h group. Furthermore, our data showed a significant decrease in gene expression for both glypican-3 and NFκB at 48 and 72h compared to the control group (p value <0.001, 0.001) respectively, with no significant effect of metformin after 24h. Also, we found a significant increase in p53 gene expression in 20μM group compared to the corresponding control cells of the same duration, while the 5μM group showed no significant difference in any duration.

Conclusion: Our research gives new insights into metformin’s anti-carcinogenic effect on HCC through its impact on LncRNA-AF085935/GPC3/MAPK pathway.

INTRODUCTION

Hepatocellular carcinoma (HCC) characterizes more than 75% of the primary liver cancers, is the sixth most predominant cancer, and is the third most frequent cause of cancer-related mortalities across the world, according to GLOBOCAN, 2020 (Chakraborty and Sarkar, 2022). HCC mortality is anticipated to double by 2040 worldwide (Foreman et al., 2018). Thus, there is high interest in finding new diagnostic and curative approaches to HCC. Risk factors that are attributed to HCC are chronic infections with hepatitis B and C viruses, factors that have been declining thanks to mass vaccination programs (Rich et al., 2020).
Diabetes, non-alcoholic fatty liver disease, alcohol, aflatoxin-contaminated food, smoking, and immune diseases are all rising risk factors (London et al., 2018). Metformin is a frontline therapy for new cases of type 2 diabetes mellitus. In addition, this inexpensive drug has been known as a strong anticancer agent with variable effectiveness among different cancers or populations (Feng et al., 2022). Metformin has been shown to lower the risk of developing HCC in diabetic patients (Cunha et al., 2019) and to improve survival in HCC cases (Schulte et al., 2019). A substantial body of evidence suggests the idea that the anti-tumoral effect of metformin is related to its ability to deactivate RAS/RAF/MAPK and PI3K/AKT/mTOR signaling pathways and down-regulate other tumor-promoting molecules (Cejuela et al., 2022).

Mitogen-activated protein kinase (MAPK) pathways include three main subfamilies (Yu et al., 2020); the extracellular signal-regulated kinases (ERKs), which include a rising member; ERK1/2 pathway, synonymously known as phospho-p44/42 MAPK pathway (Lei et al., 2017), which is usually activated by growth factors and mitogens, resulting in activating cell proliferation and endurance (Lee et al., 2020). The other subfamilies include p38 and the Jun N-terminal kinases (JNK) MAPKs. They are generally activated by cellular stress and inflammatory cytokines (Yu et al., 2020), and their role in promoting cancer is less univocal (Rovida and Tusa, 2022).

Glypican-3 (GPC3) is a cell surface proteoglycan that is abundant in the fetal liver but not in the adult liver (Zheng et al., 2022). Interestingly, GPC3 is frequently upregulated in HCC, promoting its growth and invasiveness, but is absent in non-malignant hepatic lesions (Wu et al., 2015).

In the past decade, dysregulated long noncoding RNAs (lncRNAs) have participated extensively in the pathogenesis of many cancers. LncRNA–AF085935 is an oncogene that promotes the growth of HCC cells through activating GPC3, so it is coexpressed in HCC cells and tissues (Sabry et al., 2019). To be hinted at, transcriptionally oriented, lncRNA–AF085935 is antisense to GPC3 (Lu et al., 2015). Thus, the LncRNA-AF085935/GPC3 axis has gained quite some interest as a target to be studied in HCC (AL-Noshokaty et al., 2022), (Sherif et al., 2020). It is worthy of note that the activation of the mitogenic pathway p44/42 MAPK depends on IGF-II and GPC3 (Cheng et al., 2008).

As metabolic pathways can be new therapeutic anticancer targets, we investigated the impact of low doses of metformin on the LncRNA-AF085935/GPC3/MAPK signaling pathway in HepG2 cells.

**MATERIALS AND METHODS**

**1. Cell Culture:**

HepG2 (Minnesota, USA), a human hepatocellular carcinoma cell line; was provided by VACSERA (voucher number; 00/32287) in T-75 flasks, then routinely cultured in RPMI containing glutamine 2mM (Bio west, Nampa), 10% fetal bovine serum (FBS) (PAA, Austria), and antibiotics (penicillin and streptomycin 1%) (Lonza, Belgium). The cells were incubated for 24h as a pre-culture in 5% CO2 at 37°C. Then the cells were trypsinized and then centrifuged. Cell pellets were subdivided as shown in Figure 1. Metformin (Sigma-Aldrich Co., U.S.A., CAS n1115-70-4) was freshly dissolved in 2% dimethyl sulfoxide (DMSO) as a vehicle and prepared in different concentrations (5, 10, and 20µM).
A potential Role of Metformin in Hepatocellular Carcinoma

2. Cell Viability Assay (MTT assay):
We fixed HepG2 cells into 96-well plates for 24h. Then, culture media were changed with new media supplemented with different concentrations of metformin for 24h, 48h, and 72h. For each well, the culture medium was replaced by 100µL of serum-free media and 10µL of MTT reagent and left for 4h, and then 100µL formazan (Biospes, China) replaced the medium. After incubation, we measured the absorbance in each well at 570 nm with the use of an ELISA reader (Stat Fax 2200, Awareness Technologies, USA).

3. RNA Extraction and Real-Time PCR:
RNA extraction and purification were done (Thermo Fisher Scientific Inc., Germany). At 260 and 280 nm, the total RNA yield was established using a Beckman dual spectrophotometer. Real-time PCR (StepOne, Applied Biosystem, USA) was used to assess gene expression using Vivantis, ViPrimePLUS One Step Taq RT-qPCR Green Master Mix I with ROX (SYBR Green Dye). Each target gene was normalized relative to the mean cycle threshold (CT) values of the housekeeping gene using the ΔΔCt method. Table 1 shows primer sequences for all the studied genes.

Table 1: Primers sequence of the studied genes:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence of Primer from 5′- 3′</th>
<th>F: Forward primer, R: Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glypican-3</td>
<td>F: GTCCCTGAACGCAGACTATTT</td>
<td>R: AGCTTGTGCCAGCTCTTT</td>
</tr>
<tr>
<td>lncRNA-AF085935</td>
<td>F: CAGGGGCAGCAAGGTTGTTTTC</td>
<td>R: TTTGGTGGTGCCCTGATACC</td>
</tr>
<tr>
<td>VEGF</td>
<td>F: CGGGGACCGATCTCCCTACCA</td>
<td>R: AAAATGCGGCAATCCAATTC</td>
</tr>
<tr>
<td>NFκB</td>
<td>F: CCGTGTTAATCCAAAGGCCCTA</td>
<td>R: CAGAGGGACAAACAGCAATGA</td>
</tr>
<tr>
<td>P53</td>
<td>F: GCAACGGAAACTCTCCATTTTT</td>
<td>R: CAGAGAAAGAAACGAGTAGCAGAAGA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: GTTGCTTCTGCTGACTTCAAACA</td>
<td>R: GTTTGCCTGAGCGAATTTGGCTT</td>
</tr>
</tbody>
</table>
4. Western Blot:

For all samples, the following were performed; total protein extraction (ReadyPrepTM, Bio-Rad Inc.), quantitative protein analysis (Bradford Protein Assay, Bio-Basic Inc., Canada), loading with 2x Laemmli sample buffer, electrophoresis by SDS-PAGE (FastCast™, Bio-Rad Inc.), and blotting (Bio-Rad Trans-Blot Turbo). Then the membrane was blocked at room temperature. The primary antibody of p-p44/42 MAPK (Cell Signaling Technology, #9102) was diluted and incubated overnight at 4 °C against the blotted protein. The secondary antibody (Goat anti-rabbit IgG-HRP-1mg Goat mab, Novus Biologicals) was incubated against the blotted protein for 1h at RT. A CCD camera-based imager was used to record the chemiluminescent signals (ClarityTM, Bio-Rad). Image analysis software used beta-actin (housekeeping protein) in the control sample to determine the band intensity of the target proteins.

5. Statistical Analysis:

The statistical model 18 of the SPSS software (SPSS Inc, USA) was used to plan, tabulate, and analyze the data statistically. The mean and the mean ± standard deviation (SD) were considered for quantitative data. As a measure of normality, the Kolmogorov–Smirnov test (KS) was conducted. The Mann–Whitney U test was used as a test of significance as the variables were not distributed normally. Significance was considered at p ≤ 0.05 to clarify the results of the tests of significance.

RESULTS

Microscopic pictures of cultured cells showed decreased cell viability and enhanced apoptosis of HCC upon different concentrations and durations (Fig. 2). MTT assay was utilized to assess the viability of HCC cells in vitro. After 24h, HepG2 cells supplemented with metformin showed a significant decrease in cell multiplication in all metformin-treated groups compared to the control group (p value <0.001) except the metformin 5µM-24h group (p value >0.05).

Fig. 2: Microscopic pictures of different HepG2 cancer cells.

(A, E and I): control cells cultured for 24, 48 and 72h respectively: Cells were viable, confluent (90-100%) and spindle in shape. (B) Cells treated with 5µm Metformin cultured for 24h: cells were viable, confluent (90-100%) and spindle in shape. (C) Cells
treated with 10µm Metformin cultured for 24h: cells were viable and confluent (80%).

(D) Cells treated with 20µm Metformin cultured for 24h: cells were viable but less confluent (50%) and cells were rounded in shape (indicating apoptosis).

(F) Cells fed with 5µm Metformin cultured for 48 h: cells were viable and started to be rounded in shape.

(G) Cells fed with 10µm Metformin cultured for 48 h: cells were viable, less confluent (50%) and they were almost rounded in shape.

(H) Cells fed with 20µm Metformin cultured for 48 h: All cells were rounded in shape (indicating apoptosis).

(J) Cells treated with 5µm Metformin cultured for 72h: shows viable, less confluent cells (80%) and rounded in shape.

(K) Cells treated with 10µm Metformin cultured for 72h: shows cells almost rounded in shape.

(L) Cells treated with 20µm Metformin cultured for 72h: shows unviable cells, almost rounded in shape (indicating apoptosis).

Adding to that, we noticed that the 10 and 20µM treated cells after 24 and 48h but not after 72h were significantly lower than the corresponding 5µM of the same duration (p value <0.001) (Fig. 3), suggesting that increasing the dose of metformin treatment can suppress cell proliferation rather than increasing its duration.

GPC3 showed a significant decrease in all the studied groups (compared to the control groups) for 48 and 72h (p value <0.001) but not for 24h treated cells (Fig. 4), highlighting that increasing the duration of metformin treatment can suppress the GPC3 expression. LncRNA-AF085935 expression levels exhibited a significant decrease in all treated groups (p value <0.001) except for 5µM-24h group (p value >0.05).

Fig. 3: Cell proliferation, mean ± SD levels among various studied groups. (*) Denotes a significant difference (p value <0.05) against control group of the same duration. (#) Denotes a significant difference (p value <0.05) against Metformin 5µM group of the same duration.
In addition, its expression levels in each 20μM treated cell were significantly reduced compared to the lower concentrations of the same duration (Fig. 5), suggesting that metformin can suppress LncRNA-AF085935 expression in a dose- and duration-dependent approach.

For further study of the LncRNA-AF085935/GPC3 axis, we performed a correlation between the LncRNA-AF085935 and GPC3 and found a strong positive correlation (p < 0.001) (Fig. 6).

**Fig. 4:** Glypican-3 relative gene expression. *(Mean ± SD)* among various studied groups. (*) Denotes a significant difference (p value <0.05) against control group of the same duration.

**Fig. 5:** LncRNA-AF085935 relative gene expression *(Mean ± SD)* among various studied groups. *p value* <0.05 was significant. (*) Denotes a significant difference against control group of the same duration. (#) Denotes a significant difference versus Metformin 5μM group of the same duration. ($) Denotes a significant difference versus Metformin 10μM group of the same duration.
Concerning MAPK protein level, we have measured the ratio between the phosphorylated p44/42 and the total p44/42 and found a significant decrease in the protein level in treated groups (versus the control group) (p value <0.0001) except for 5µM-24h group (p value >0.05). It was noted that the 10µM-24h and 20µM-24h groups showed less significance (p values 0.02, and 0.001) respectively (after comparing the corresponding concentrations of higher duration with their control values), supporting the duration-dependent effect of metformin on MAPK (Fig. 7).

**Fig. 6:** Correlation between Glypican-3 gene expression and LncRNA AF085935 expression among various studied groups.

**Fig. 7:** Western blotting analysis of p-p44/42 MAPK and the total p44/42 MAPK protein levels (Mean ± SD) among various studied groups. *p value <0.05 is significant. (*) means a significant difference versus control group of the same duration. (#) means a significant difference versus Metformin 5µM group of the same duration.
VEGF is the principal driver of angiogenesis. In our study, we found a significant decrease in VEGF relative gene expression in all metformin-treated groups (p value <0.001) except for the metformin 5µM-24h group (p value = 0.9). The latter showed significantly higher VEGF gene expression relative to the 20µM-24h group (Fig. 8).

![Fig. 8: VEGF gene expression (Mean ± SD) among various studied groups.](image)

*p value <0.05 was significant. (*) means a significant difference versus control group of the same duration. (#) means a significant difference versus Metformin 5µM group of the same duration.*

Regarding apoptosis, we assessed p53 gene expression and found a dose- and time-dependent significant increase in P53 gene expression in the 20µM treated cells compared to the corresponding control cells of the same duration. This defined group also showed a significant increase if compared to the correspondingly lower concentrations only after 48 and 72h (Fig. 9). It is worthy of note that the 5µM group showed no significant difference in any duration.

![Fig. 9: P53 gene expression (Mean ± SD) among various studied groups](image)

*p value <0.05 was significant. (*) Denotes a significant difference versus control group of the same duration. (#) Denotes a significant difference versus Metformin 5µM group of the same duration. ($) Denotes a significant difference versus Metformin 10µM group of the same duration.*

As regards nuclear factor κB (NFκB) gene expression, our study showed a significant decrease in NFκB gene expression in all groups treated with metformin compared to the control group (p value <0.001) after 48 and 72 h only (Fig. 10).
DISCUSSION

Despite treatment breakthroughs, hepatocellular carcinoma (HCC) remains among the most lethal malignancies globally. The antidiabetic multitarget drug metformin is gaining more attention for its anticancer effects against HCC development. Following intestinal absorption, metformin in the most commonly prescribed doses of 1 to 2g/day leads to plasma concentrations of 10 to 40µM (Kajbaf et al., 2016). However, most studies dealing with metformin in cancer were conducted at 10- to 100 times higher concentrations than maximally practicable therapeutic ones (He and Wondisford, 2015).

Based on our curiosity about metformin, the current study was designed to explore whether therapeutic doses of metformin (5, 10, and 20µM) at different durations (24, 48, and 72h) could affect LncRNA-AF085935/Glypican-3 and MAPK pathway in HCC in vitro.

Regarding cell proliferation, our results exposed a significant decrease in all metformin-treated groups except the metformin 5µM-24h group, supporting the dose- and time-dependent effects of metformin on cell proliferation. We share consistent results of these low-dose effects with a few other studies (Sabry et al., 2019), (Sun et al., 2016), (Zhang et al., 2017).

Similar studies, but with higher concentrations of metformin (in millimoles) applied to HCC, also showed harmonious results (Cai et al., 2013), (Miyoshi et al., 2014), (Ferretti et al., 2019), (Tawfik et al., 2022). Other studies using metformin on other types of cancer cell types confirm the same concept, including surgically resected glioblastoma tissue samples (Guarnaccia et al., 2022) and human gastric cancer cells (Lu et al., 2019).

Concerning the LncRNA-AF085935/GPC3 axis, both parameters showed significantly independent decreased levels in the treated groups, especially after 48 and 72h and they revealed a strong positive correlation with one another. In agreement with us, Sabry et al., 2019 determined a significant decrease in both GPC3 protein expression level and LncRNA-AF085935 expression in HepG2 cancer cells after supplementation with EGCG and metformin, illustrating that metformin is more effective than EGCG on this lncRNA. Those can support Lu et al., 2015, who assumed that LncRNA-AF085935 could be a possible biomarker for HCC. The effect of metformin on decreasing GPC3 expression may aggravate the inhibitory effect of metformin on insulin-like growth factors (IGFs) and their binding proteins as GPC3 induces...
oncogenicity by preventing IGF-1R degradation (Cheng et al., 2017).

Concerning MAPK protein level, our study revealed a significant decrease in p-p44/42 MAPK protein level in treated groups except for 5µM-24h group and less significance in the 10µM-24h and 20µM-24h groups, supporting the duration-dependent effect of metformin on p-p44/42 MAPK. GPC3 may enhance p44/42 MAPK activation, promoting the epithelial-mesenchymal transition (EMT) of HCC cells (Wu et al., 2015). A mechanism that clarifies the GPC3/MAPK link is that GPC3 interacts with FAT1, an integral protein on the cell surface of HCC cells, and acts concomitantly to regulate the expression of EMT-related genes Snail, Vimentin, and E-Cadherin and promote HCC (Meng et al., 2021). GPC3, in addition, has the ability to bind to IGF-II along with IGF-1 receptors (IGF-1R), triggering phosphorylation of IGF-1R as well as the subsequent downstream signaling molecule p44/42 MAPK (Cheng et al., 2008).

Blankly, GPC3 had another behavior in breast cancer, as it was found that GPC3 reverses the EMT, inhibits metastasis, and enhances dormancy of cancer cells by activating p38 MAPK and that p-p44/42/phospho-p38 ratio is lower in GPC3 re-expressing cells (Guerenño et al., 2020). The hierarchical relationships among different MAPK pathways and GPC3 remain to be determined.

The anti-carcinogenic effect of metformin on MAPK follows the same line as NFκB gene expression, which revealed a significant decrease in all groups treated after 48 and 72h. That results support a previous report (Hsieh et al., 2014) where metformin inhibited the invasion of human HCC cells and boosted the chemosensitivity to sorafenib via downregulating the ERK/JNK in an NF-κB-based pathway. In addition, millimolar concentrations of metformin attenuate p44/42 phosphorylation/anti-apoptotic signaling as previously reported in gastric cancer (Lu et al., 2019), MCF-7 breast cancer cell line (Malki and Youssef, 2011), papillary thyroid carcinoma cells (García-Sáenz et al., 2022), ovarian cancer cells (Dang et al., 2017), and A549 lung cancer cells (Dong et al., 2020), (Zhang et al., 2018). Contrastingly to our results, He et al., 2021 found that metformin can affect MAPK signaling via inhibiting stimulation of p38 MAPK and JNK MAPK but not via p44/42 MAPK and rescue insulin secretion in type 2 diabetes. They used both in vivo diabetic rat models and in vitro rat pancreatic β-cells. This conflict may be caused by the use of different species or by the fact that the effects of metformin on MAPK signaling may differ in cancers.

Metformin decreases the vascularization of HCC cells in a dose- and duration-dependent way in our study, as we found a significant decrease in VEGF gene expression in all treated groups except 5µM-24h group, inhibiting its angiogenic effect. This result supports the previous results of Sabry et al., 2019 on metformin-treated HepG2 cells and Dang et al., 2017 after applying higher concentrations of metformin to the human ovarian HO-8910 cancer cell line.

Regarding apoptosis, we found a significant increase in P53 gene expression, especially with 20µM after 48 and 72h. Metformin allows augmentation of p53 and its putative stabilization through its action on adenosine monophosphate-activated protein kinase (AMPK) (Ferretti et al., 2019) as metformin is a well-known inhibitor of complex I of the respiratory chain, resulting in consequent activation of AMPK (Skuli et al., 2022). This effect can be augmented if combined with antifolates (Miyoshi et al., 2014) and can be mediated through miR-23a, a tumor suppressor induced by metformin (Sun et al., 2016).

Conclusion

Taken together, these studies and our present findings suggest that low doses of metformin (10 and 20µM) may have the potential to suppress HCC by decreasing viability and division of cancer cells, downregulating MAPK, Glypican-3, LncRNA-AF085935, VEGF, and NF-κB gene expression, and upregulating P53 gene expression, while the effect of metformin
5μM, when applied for a short duration (24h) is still a subject of debate. Findings that need further investigation by functional assays in multiple cell lines or through in vivo studies in the future

**Statements & Declarations:**

**Funding:** This work was financially supported by the Faculty of Medicine, Cairo University, Egypt.

**Competing Interests:** The authors have no relevant interests to disclose.

**Authors Contributions:**

Investigation: Radwa Taha, Mona Said, Naglaa Fathy.

Project administration and resources: Dina Sabry

Software: Mona Said.

All authors contributed to the study conceptualization, design, validation, writing, editing, and approval of the final manuscript.

**Ethics Approval:** The study was approved by the Faculty of Medicine, Cairo University, Egypt.

**Data Availability:** All data engendered during this work are encompassed in this manuscript.

**REFERENCES**


Sherif IO, Al-Mutabagani LA, Sabry D, Elsherby NM (2020). Antineoplastic Activity of Chrysin against Human Hepatocellular...


موجز:

LncRNA-AF085935 في سرطان الخلايا الكبدية: دراسة في المعمل لمسار Glypican-3 / MAPK

المؤلف

دور محتمل للميتفورمين في سرطان الخلايا الكبدية: دراسة في المعمل لمسار Glypican-3 / MAPK

ملحوظة:

1. الكيمياء الحيوية الطبية والبيولوجيا الجزيئية - كلية الطب - جامعة القاهرة - مصر
2. الكيمياء الحيوية الطبية والبيولوجيا الجزيئية - كلية الطب - جامعة بدر - مصر

ملحوظة:

بعد سرطان الخلايا الكبدية عن أكثر أنواع السرطانات انتشرت في العالم حيث يعد سرطان الخلايا الكبدية واحدًا من أكثر أنواع السرطانات انتشرت في مصر وعادةً ما يكون سبب تلف الكبد بسبب العوامل الفيروسية خصوصاً عدوى الفيروس البستاني الكبير. بسبب انتشار الكبد والمرفقات في جميع أنحاء العالم، يتم تقديم الكثير من الدراسات في تشخيص وعلاج سرطان الخلايا الكبدية.

وقد تظهر العناصر التي يتم تشخيصها بطرق عديدة، في حين أن العناصر الأخرى لم تكن متوفرة.

سير مرض سرطان الخلايا الكبدية لا يزال صعبًا، ويرجع ذلك إلى حد كبير إلى المراحل المتقدمة في الوقت الذي يتم فيه تشخيصه. ومنذ الإفلاس والانتشار السريع للمرض، يتم توظيف الاستراتيجيات الخاصة جيدة.

تستخدَم عقار الميتفورمين كخيار أول لعلاج النوع الثاني للسكري، مع توفير appréciة عن فائدته للوقاف.

يهدف إلى تحقيق أشخاص من عقار الميتفورمين على الانتشار وموت الخلايا البرمجية وتقليل الإزدحام في سرطان الخلايا الكبدية.

أجريت الدروس في المختبر على خلايا سرطان الخلايا الكبدية بتركيزات مختلفة من عقار الميتفورمين (10-20 ميكرو مولر) على فترات زمنية مختلفة (48 و72 ساعة) لقياس جوانب التعبير المكسيكية - التحكم غير المعالجة. التعبير المكسيكية: التحكم غير المعالجة.

تتم تقسيم الدراسة إلى أربعة مجموعات: المجموعة الأولى (الضبابية)، وتضم خلايا الكبد السرطانية الغير معالجة المستمرة لمدة 48 و72 ساعة. المجموعة الثانية: تضم خلايا الكبد السرطانية المعالجة بتكرار 5 ميكرو مولر من عقار الميتفورمين مستمرة لمدة 24-72 ساعة. المجموعة الثالثة: تضم خلايا الكبد السرطانية المعالجة بتكرار 10 ميكرو مولر من عقار الميتفورمين مستمرة لمدة 24-72 ساعة. المجموعة الرابعة: تضم خلايا الكبد السرطانية المعالجة بتكرار 20 ميكرو مولر من عقار الميتفورمين مستمرة لمدة 24-72 ساعة.

تتم عمل الاباح لمجموعات الدراسة:

1. قياس معدل تكاثر خلايا الكبد السرطانية باختبار الام تي.
2. قياس معدل التعبير الجيني الكمي لكل من الجينات الثلاثية (هيجينك3 بحمض الريبونوكليك الطويل الغير مشفر) في مجموعة المحمولة (53950) وعمال نظام بطاقة الوراثة الخلاياية في وحدة العرض العام كلي (بطينية متسلسلة). تقييم العلاقة بين التعبير الجيني الكمي في الجينات الثلاثية (هيجينك3 بحمض الريبونوكليك الطويل الغير مشفر) في مجموعة المحمولة (53950) وعمال نظام بطاقة الوراثة الخلاياية في وحدة العرض العام كلي.
3. قياس معدلات تصنيف السرطانات في جميع المجموعات المعالجة بعقار الميتفورمين مستمرة في المجموعة الثلاثية المختلفة (48 و72 ساعة).

نتائج الدراسة:

1. نقص المحلول في معدل تكاثر خلايا الكبد السرطانية باختبار الام تي في المجموعة الثلاثية المختلفة (48 و72 ساعة). من الصعب استثناء تكاثر 5 ميكرو مولر (الذي لم يظهر دالالة في أقصى 72 ساعة) في كل المجموعات المعالجة بعقار الميتفورمين مقارنة بالocumentation}

تلتئم الدكتور محمد علي

2. هناك ارتفاع ملحوظ في التعبير الجيني لجين الجلابكن في جميع المجموعات المعالجة بعقار الميتفورمين مقارنة بالمستقبلة البديلة في 72 و48 ساعة. في حين لا يوجد أي فرق كبير يذكر في 24 ساعة.
هناك انخفاض ملحوظ في التعبير الجيني لحمض الريبونوكليك الطويل الغير مشفر أ.آ. ف 539580 في كل المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في المدد الزمنية الثلاثة المختلفة (24، 48، 72 ساعة) باستثناء ادنى تركيز (5 ميكرومولار) الذي لم يظهر دالة في أقصر مدة وهي 24 ساعة. 

هناك انخفاض ملحوظ في التعبير الجيني لبروتين في الفسفوري p44 / p42 وكذلك عامل نمو بطانة الأوعية الدموية في كل المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في المدد الزمنية الثلاثة المختلفة (24، 48، 72 ساعة) باستثناء ادنى تركيز (5 ميكرومولار) الذي لم يظهر دالة في أقصر مدة 24 ساعة. 

هناك زيادة ملحوظة في التعبير الجيني لجين بي 53 في المجموعات المعالجة بتركيز 10 و20 ميكرومولار واضحة مع زيادة المدة ولكن لم تظهر المجموعة المعالجة بتركيز 5 ميكرومولار مثل هذه الزيادة. هنالك انخفاض ملحوظ في التعبير الجيني للعامل النووي كابا بي في المجموعات المعالجة بعقار الميتفورمين مقارنة بالمجموعة الضابطة في (48 و72 ساعة) في حين لا يوجد أي فرق يمكن ذكره في 24 ساعة. هناك ارتباط جيد جدا بين مستوي التعبير الجيني لجين جلابكن 3 وتعبير الجيني لحمض الريبونوكليك الطويل الغير مشفر أ.آ. ف 539580 / جين جلابكن 3 / بروتين p44 / p42

الخلاصة: ترجح الدراسة الحالية أن عقار الميتفورمين له تأثير علاجي على سرطان الخلايا من خلال حمض الريبونوكليك الطويل الغير مشفر A.آ. ف 539580 / جين جلابكن 3 / بروتين p44 / p42.